DNA Casework Unit
Procedures for Extraction of DNA with QIAamp Purification

1 Scope

These procedures describe the process for chemical digestion and purification of deoxyribonucleic acid (DNA) from body fluids and tissues (e.g., buccal swabs, blood).

2 Equipment/Materials/Reagents

Equipment/Materials

- General laboratory supplies (e.g., tubes, pipettes, centrifuge)
- Extraction basket, Costar Spin-X or equivalent
- Qiagen® QIAamp Mini Spin columns
- Qiagen® collection tubes, or equivalent
- Sonicator

Reagents

- Ethanol (EtOH), absolute
- Stain Extraction Buffer (SEB)
- Dithiothreitol (DTT), 5M solution
- Proteinase K (ProK), 20mg/mL
- Qiagen® QIAamp DNA Mini Kit
  - Qiagen® Buffer AL
  - Qiagen® Buffer AW1
  - Qiagen® Buffer AW2
- Water, Reagent Grade or equivalent
- 1% Sodium Dodecyl Sulfate (SDS), if necessary
- Xylene, if necessary
- Xylene substitute, if necessary

3 Standards and Controls

At least one extraction control (i.e., reagent blank [RB]) must be processed in parallel with each extraction batch.

For evaluation of the extraction controls, refer to the appropriate interpretation procedure of the *DNA Procedures Manual*.

4 Procedures

Refer to the DNA Procedures Introduction (DNA QA 600) for applicable general precautions and cleaning instructions.

Supplies typically needed for processing 1 sample and 1 RB (adjust for batches):

- EtOH (~1 mL), AW1 (~1.2 mL), AW2 (~1.2 mL), AL (~800 µL)
- 4 - 1.5 mL tubes
- 2 - QIAamp columns with collection tubes
- p2, p20, p200, p2000 pipettes

Tissue samples may be rinsed in any combination of the following: water, 1% SDS, xylene, xylene substitute, and/or EtOH prior to extraction based on the nature of evidence.
Record cleaning method(s) used in case notes. For additional guidance on paraffin embedded tissue, refer to the DNA collection procedure (DNA 201).

For bloodstains, measure and record the stain size in the case notes.

In general, refer to the Sampling section of this procedure and/or DNA collection procedure (DNA 201) for guidance on sample size and sample collection.

4.1	Enter appropriate barcodes. Prepare SEB + DTT by adding 7.8 µL 5M DTT to 1 mL SEB.	

4.2	Add 300 µL of SEB+DTT and 2 µL ProK to each sample and RB tube. Vortex briefly and incubate with agitation at 56ºC for 2 hours to overnight.	

4.3 QIAamp DNA Mini Kit Purification

4.3.1	Remove tubes and pulse spin. If appropriate, transfer cutting, swab, or remaining undigested sample into a basket. Place basket into tube containing liquid extract and pulse spin. Remove and discard basket.	

4.3.2	Add 300 µL Buffer AL. Invert mix. Incubate at 70ºC for 10 minutes.	

4.3.3	Pulse spin. Add 400 µL EtOH. Vortex mix. Pulse spin.	

4.3.4	Add 500 µL of sample or RB to the corresponding QIAamp column within a collection tube. Spin at 8000 X g for 1 minute. Discard waste and return column to collection tube.	

4.3.5	Add remaining ~500 µL of sample or RB. Spin 8000 X g for 1 minute. Discard waste and return column to collection tube.	

4.3.6	Add 500 µL Buffer AW1. Spin 8000 X g for 1 minute. Discard waste and return column to collection tube.	

4.3.7	Add 500 µL Buffer AW2. Spin 13000-14000 X g for 3 minutes. Discard waste and return column to collection tube.	

4.3.8	Spin 13000-14000 X g for 1 minute. Transfer column to a 1.5 mL tube for elution.	

4.3.9	Add 105 µL Water. Incubate at 70ºC for 5 minutes. Spin 8000 X g for 1 minute.	

Samples may be eluted in a different final volume at Examiner's discretion. If a different volume is used, record volume in case notes.

Known and tissue samples do not require mito preamplification quantitation (qPCR) and may proceed to amplification. Store extracted DNA at 4ºC or colder.

Samples may be diluted in water prior to amplification. Record any dilutions in case notes. The general guide for dilution ratios are:

Buccal swab: 1:50
Blood stains: Heavy: 1:50 Light: 1:10 Gauze: 1:10
Liquid blood: 1:50
Tissue samples: 1:10

5 Sample Selection

There is a reasonable assumption of homogeneity and no sampling plan is needed. In general, the size of a sample used for extraction is as listed below. The amount of sample may vary based upon sample condition.

Buccal swab: ~ ¼ - ½ of a swab tip
Blood stain: ~3 mm x 3 mm cutting of stain
Liquid blood: ~5 µL
Tissue: ~3 mm x 3 mm x 3 mm portion

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

The quantity and quality of the DNA present within any biological material ultimately determines if a DNA extraction is successful.

9 Safety

9.1 All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material. Follow the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual*.

9.2 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

9.3 Procedural Specific Chemical Hazards:

- The sample-preparation waste contains guanidine hydrochloride from Buffers AL and AW1, which can form highly reactive compounds when combined with bleach.

- Proteinase K can be irritating to mucous membranes. Use eye protection when handling.
- Xylene is an irritant and is toxic. Its use will be confined to a chemical fume hood whenever possible.

10 References

FBI Laboratory Safety Manual

FBI Laboratory Quality Assurance Manual

FBI Laboratory Operations Manual

DNA Procedures Manual

QIAamp® DNA Mini and Blood Mini Handbook, QIAGEN®.

Rev. #	Issue Date	History
0	02/05/16	Reformatted from Mitochondrial DNA Analysis Laboratory Procedures. Added additional information for clarity. Removed instruction to add sample to tube containing reagent allowing for collection of sample prior to initiating extraction. Allowed for using the same QIAamp column collection tube. Eliminated requirement to measure final extract volume

Approval

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